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Whole-Genome Sequences of Thirteen Isolates of *Borrelia burgdorferi*▽†

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***Borrelia burgdorferi* is a causative agent of Lyme disease in North America and Eurasia. The first complete genome sequence of *B. burgdorferi* strain 31, available for more than a decade, has assisted research on the pathogenesis of Lyme disease. Because a single genome sequence is not sufficient to understand the relationship between genotypic and geographic variation and disease phenotype, we determined the whole-genome sequences of 13 additional *B. burgdorferi* isolates that span the range of natural variation. These sequences should allow improved understanding of pathogenesis and provide a foundation for novel detection, diagnosis, and prevention strategies.**

Lyme disease is the most frequent tick-borne disease in North America and Europe (3, 16, 17). There are multiple variants of *B. burgdorferi* (1, 7, 15, 20, 21), the causative agent, but questions remain about how their variation correlates with different clinical manifestations. Whole-genome sequencing (WGS) can orient approaches to diagnostics and vaccines and help avoid potential host cross-reactivity. Improved diagnostics are needed because the best clinical sign, the erythema migrans skin rash, does not always occur. Diagnostic assays and vaccines (18) have been less than satisfactory. However, these were developed before WGS of microbes and the human genome. This project was stimulated by the initial finding of genotypes of *B. burgdorferi* associated with invasiveness/dissemination (15). This has been substantiated (7, 21).

The sequencing of strain B31 (6, 8) has accelerated progress in Lyme disease research. We sequenced 13 additional isolates, chosen to cover a large fraction of the genetic and geographic diversity and obtained from humans and other natural hosts (Table 1).

These genomes were sequenced by the random shotgun method as described previously, using Sanger DNA sequencing to an estimated 8-fold coverage (12). Approximately 10,000 and 6,000 successful reads for the small and medium insert plasmid libraries, respectively, were sequenced, representing a total of about 14 Mbp of sequencing data for each. All plasmids were sequenced to closure unless noted otherwise (see Table S1 in the supplemental material). Genome annotation was performed using the JCVI Prokaryotic Annotation Pipeline (www.jcvi.org/cms/research/projects/prokaryotic-annotation-pipeline/overview/).

The B31 sequence showed that *B. burgdorferi* has many more replicons (DNA molecules) than other bacteria. Besides its 910-kbp linear chromosome, strain B31 has been shown to have 12 linear and 10 circular plasmids (5), expanding observations (2, 10) indicating that *Borrelia* bacteria universally harbor numerous plasmids, many essential for survival of the bacteria in mice and/or ticks (4). The newly sequenced genomes contain a total of 17,084,900 bp, averaging 1,314,223 bp/genome. Each strain carried between 13 and 21 plasmids (239 plasmids were sequenced, about half predicted to be linear replicons). At least 9 new plasmid types not in B31 were identified. Many plasmids underwent substantial rearrangements in different lineages. The linear chromosomes are very stable, with little variation among isolates. With the exception of a few differences at their right ends, the gene content of the chromosomes is essentially identical. Contrary to previous assumptions that genetic changes occurred only by slower point mutations, our initial WGS comparison of 4 strains showed that closely related *B. burgdorferi* strains frequently and more rapidly than by point mutation undergo horizontal exchange of genetic information (14). Evidence of this is also found in the newer genomes sequenced in this work.

The genetic diversity of *B. burgdorferi* appears to be maintained in part by neutral and adaptive processes, such as resistance to host immune defense mechanisms and host preferences (4, 9). Key questions remain on the genomic basis of these intra- and interspecific variations, particularly those associated with host resistance, high-frequency proliferation in wildlife populations, and invasiveness in humans.

Our long-range objectives are to develop a pangenomic picture of *B. burgdorferi* diversity (13) and to understand how the variations influence pathogenicity. We believe solutions for

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TABLE 1. *B. burgdorferi* isolates used in this study

Borrelia burgdorferi isolate[a]	rRNA IGS1 lineage[b]	MLST type[b]	OspC type[b]	Chromosome sequencing status	Total no. of bp sequenced	No. of contigs in chromosome (no. of bp)	No. of plasmids[c]	Genome Project ID[d]
64b	3	7	Ba	Draft	1,485,884	6 (905,796)	18	28633
72a[f]	26	14	G	Draft	1,267,789	6 (905,797)	13	21003
94a	8	18	U	Draft	1,295,451	9 (906,731)	14	20999
118a	20	34	J	Draft	1,453,013	8 (903,362)	19	21001
156a	12	4	Hb	Complete	1,469,834	1 (908,814)	20	19835
297	2	3	K	Not sequenced	508,697		20	39123
29805	6	12	M	Draft	1,344,204	38 (887,933)	15	28621
B31[e]	1	1	A	Complete	1,522,832	1 (910,724)	21	3
Bol26	3		S	Draft	1,321,434	4 (909,216)	13	19837
CA-11.2A	19	70	Db	Draft	1,294,354	14 (907,566)	13	28629
JD1	24	11	C	Complete	1,531,287	1 (922,801)	20	39121
N40	9	19	E	Complete	1,339,552	1 (902,191)	17	39119
WI91-23	7	71	Ia	Draft	1,427,907	31 (896,127)	21	28627
ZS7	16	20	Bb	Draft	1,345,494	1 (906,707)	14	19839

[a] Table S1 in the supplemental material gives the origins and sources of these strains.
[b] According to Travinsky et al. (20) and references therein.
[c] Nearly all plasmids were sequenced to closure; the few remaining in draft status are noted in Table S1 in the supplemental material.
[d] The Genome Project ID retrieves the data from each genome in the NCBI Entrez Genome Project Database (www.ncbi.nlm.nih.gov/genomeprj/GPID).
[e] The sequence of the genome of strain B31 was previously reported and is included here for comparison. Its bp value includes terminal bp determined by Fraser et al. (8), Zhang et al. (22), Huang et al. (11), and Tourand et al. (19). We independently determined the sequence to the tips of the B31 lp54 plasmid, and our results are in agreement with those of Tourand et al. (19); (J. Aron, S. Casjens, and W. M. Huang, unpublished). The B31 lp28-1 length was assembled from several published sources.
[f] This is not the same as isolate 72a reported by Qiu et al. (14). That original 72a strain is apparently lost.

many of the problems associated with Lyme disease will come from scientific information, beginning with comparative genomics of this organism. Sequencing is a superb discovery tool whose greatest impact is realized when additional biology can implemented. Information from WGS of these well-characterized strains should provide a foundation for new hypotheses on the pathogenesis of Lyme disease and rational diagnostics and vaccines.

Nucleotide sequence accession numbers. These sequences have been deposited in GenBank, and their Genome Project ID numbers and accession numbers are listed in Table 1 and in Table S1 in the supplemental material, respectively.

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